

The Association between Occupational Exposure to Pesticides
and Cardiovascular Disease Incidence:
The Kuakini Honolulu Heart Program

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Zara Kunhausen Berg

Dissertation Committee:

Dr. Beatriz Rodriguez, Chairperson

Dr. Al Katz

Dr. James Davis

Dr. Kamal Masaki

Dr. Robert Cooney, University Representative

Abstract

Previously, Kuakini Honolulu Heart Program researchers reported that occupational exposure to pesticides is significantly associated with total mortality.

Objective: The current study examines occupational exposure to pesticides on the job in relation to incident cardiovascular diseases (CVD), that is, coronary heart disease (CHD) incidence and cerebrovascular accident (CVA) incidence, combined. **Methods:** Using the OSHA exposure scale as an estimate of exposure, statistical analyses were performed using a cohort of 7,557 Japanese-American men from The Kuakini Honolulu Heart Program.

Results: In the first 10 years of follow-up of the cohort, a positive correlation was observed between age-adjusted CVD incidence and pesticide exposure with a p-value of 0.021. This relationship remained significant after adjustment for other CVD risk factors. No significant association for coronary heart disease or stroke incidence and pesticide exposure was observed when examined separately, possibly due to a smaller number of events. The biochemical mechanisms leading to CVD and associated risk factors will be discussed.

Conclusion: These results are novel, as the association between occupational exposure to pesticides and cardiovascular disease incidence has not been examined previously in this cohort. These findings may contribute to our understanding of the role of occupational exposure to pesticides plays in the development of cardiovascular diseases.

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List Of Acronyms

1. ACHE – acetylcholinesterase
2. BMI – body mass index
3. BPA – biphenyl A
4. BuCHE – butyrylcholinestrace
5. CDC – center for disease control and prevention
6. CHD – coronary heart disease
7. CVA – cerebro-vascular accident
8. CVD – cardiovascular disease
9. DBP – diastolic blood pressure
10. DDD – dichloro-diphenyl-dichloro-ethane
11. DDE – dichloro-diphenyl-dichloro-ethylene
12. DDT – dichloro-diphenyl-trichloro-ethane
13. EKG - electrocardiogram
14. EPA –Environmental Protection Agency
15. HDL – high – density lipoprotein
16. HHP - Honolulu Heart Program
17. HAAS – Honolulu–Asia Aging Study
18. LDL – low-density lipoprotein
19. NIOSH – National Institute for Occupational Safety and Health
20. OSHA – Occupational Safety Health Administration
21. PCB – polychlorinated biphenyls
22. PCDD – polychlorinated dibenzodioxins
23. PCDF – polychlorinated dibenzofurans
24. PEL – permissible exposure limit
25. PON1 - paraxonase 1 gene
26. POP – persistent organic pollution
27. PPE – proper protective equipment
28. ROS – radical oxidation species
29. SAS – statistical analysis systems
30. SBP – systolic blood pressure
31. TCDD – tetrachlorodibenzodioxin
32. TWA – time-weighted average
33. VA – Veterans Affairs
34. VLDL –very-low density lipoprotein

Chapter One: Introduction

In 2008, coronary heart disease was the number one cause of death, and stroke was the third most common cause of death in the United States (1). This public health issue is significant because the United States has an increasing elderly population. Some of these older individuals may have been exposed to harmful chemicals on the job at a time when EPA exposure limits did not exist and subjects did not have proper protective equipment during occupational exposure to these chemicals. This research will examine the effect of occupational exposure to pesticides in middle age and its effect on men as they become older. Thus far, limited research has been done on the effects of occupational exposure in middle aged men over a 30 year follow-up in relation to cardiovascular diseases.

Death from occupational exposure in the United States is the eighth leading cause of death right below diabetes, which includes coronary heart disease from occupational exposure, thus it is suspected that cardiac related death from occupational exposure is one of the contributing causes (2). The goal of this project is to establish a possible association between occupational exposure with incidence of CVD, in order to prevent possible CVD morbidity related to occupation, with the purpose of reducing the social and economic burden of health care costs in this country.

This proposed study is different than prior research because instead of focusing on mortality of various cardiovascular diseases, it will focus on the incidence of the disease. The original goal of this project was to measure the potential effect of pesticides, solvent and metal exposure in

relation to cardiovascular disease incidence. However, since no association was found for solvents and metals, the goal of the study was revised to focus on pesticides only. In some studies, acute exposure to pesticides increases the risk of mortality rates from circulatory diseases, specific cancers, and from all occupational exposure causes combined (1). The scientist found no association between handgrip strength and developing arthritis in relation to occupational exposure to pesticides, solvents and metals (1,3).

Another reason for the significance of this study is due to the new fields of environmental cardiology and occupational epidemiology of the 21st century, which faces many challenges due to climate change and various environmental pollutants. Environmental cardiology combines the study of environmental exposure (air particulate matter, inhalation, absorption of chemical through the skin via the surface area in both skin and lungs) and predetermined public health intermediate risk factors (triglycerides, glucose, total cholesterol, BMI.) in relation to susceptibility to disease. Previous scientific publications on various occupational exposure have contributed noise greater than 85 db, job strain, increased work load leading to stress lead to circulatory diseases. In addition, occupational and environmental medicine studies agreed that factors in the American diet and changes in climate change with the combination of chemical occupational exposure lead to the development of various diseases (2,4). The association between environmental exposure to these chemicals, and inflammation from oxidative stress and current medical risk factors for CVD, CHD, and stroke has not been well established yet, which could partially be due to a combination of environmental and occupational exposures. As the environmental and occupational exposure increases and the average age of the baby boomers increases, America will have a huge economic and public health burden of disease.

Chapter 2: Does occupational exposure to pesticides over time lead to cardiovascular disease mortality and morbidity?

Health agencies worldwide state that cardiovascular disease is the number one cause of death worldwide (1). Cardiovascular disease (CVD) affects the heart and circulatory system; circulatory diseases include coronary heart disease (CHD), cerebrovascular disease, cerebral vascular accident (CVA), peripheral arterial disease, and rheumatic heart disease. Stroke is the third leading cause of death in developing nations (2). According to the Center for Disease Control and Prevention in 2008, coronary heart disease was the number one cause of death, and stroke the third-highest cause of death in the United States. The public health issue of cardiovascular disease is especially significant in the United States as it has an increasing elderly population. In addition to established cardiovascular disease risk factors, it is suspected that numerous occupational exposures may contribute to disease development. This elderly population lived and worked during a time when Occupational Safety & Health Administration (OSHA) and the Environmental Protection Agency (EPA) exposure limits were not yet established and proper protective equipment (PPE) was not yet in use, leading to substantial occupational exposure to pesticides. Death from occupational exposure to pesticides in the United States is the 8th leading cause of death, just lower than diabetes, and includes coronary heart disease from occupational exposure (3). It is suspected that cardiac-related deaths from occupational exposure are high (4,5,6,7). Most studies on occupational exposures to pesticides and their relationship with CHD, CVD, and CVA (stroke) have been conducted using risk assessment, with several factors taken into consideration: noise exposure; social and economic status; stress; particulate matter; radiation, and combustion chemicals. A number of studies have analyzed pesticide exposure in relation to the incidence of cancer and neurological disorders (8).

The majority of the studies that reviewed occupational pesticide exposure and its link with CHD, CVA, or CVD, dealt primarily with various causes of mortality, especially cancer. Cancer resulting from occupational exposure to pesticides at work develops at a higher rate than cardiovascular diseases during the lifetimes of those affected.

A number of journal articles discuss various neurological diseases of the peripheral and central nervous system that can lead to dementia, Alzheimer's disease, and Parkinson's disease. Some investigations report destruction of cerebral vascular flow in the brain from pesticide exposure, which can potentially lead to stroke (9).

Pesticides are neurotoxins that were originally invented in the early 1860s for chemical warfare but were not utilized until World War I (9). During World War II, there was an excess of neurotoxic chemicals produced for the sole purpose of chemical warfare. After the war, with a huge surplus of these chemical agents, a new market was created and the chemical weed-killing business became a booming industry. Although in the 1970s, persistent organic pollutants (POPs) like DDT were banned in the United States, these chemicals are still manufactured in the US and sold worldwide. To date, spraying DDT is still the best practice in tropical areas for decreasing occurrences of malaria and other tropical diseases. Although the majority of POPs categorized as the 'deadly dozen' are not manufactured anymore, these chemicals are still found throughout the environment through the grasshopper effect. Global distillation or the grasshopper effect is the geochemical process by which certain chemicals, most notably persistent organic pollutants (POPs), are transported from warmer to colder regions of this planet, especially the poles.

In some industries, using pesticides is still the cheapest and most efficient method for the manufacturing process for many industrial and agricultural tasks (degreasing machinery, farming, pest reduction, insect repellent).

Of further significance in this literature review is the emergence of the relatively new fields of environmental cardiology and occupational epidemiology. Environmental cardiology is the study of co-exposure to environmental toxins (e.g. air particulate matter, pesticides, metal exposure) and predetermined public health risk factors (BMI, weight, etc.) in relation to susceptibility to diseases. Several factors such as noise exposure greater than 85 decibels, job strain, and an increase in workload on individuals, factors from climate change and change in the American diet have been associated with susceptibility to disease (10,11). Science is just starting to understand free radical oxidation (ROS) damage and inflammation in relation to causing diseases. ROS and inflammation could partially be due to a combination of environmental and occupational exposures that affect intermediate risk factors (e.g., body mass index, cholesterol, glucose, triglycerides) for CVD. Now technology is being developed to assess biomarkers and measure chemical exposure in small quantities that was not possible before (12). As the elderly population increases, along with the average age of the baby boomer generation in America, this may result in a significant economic burden and require major public health reform. By analyzing studies on the mortality of diseases of the cardiovascular system associated with occupational exposure, there is a possibility that additional prevention methods could be found and be promoted in health care policy.

Materials and Methods

The database used to find articles was PubMed through the University of Hawaii – Manoa subscription and the National Institute for Occupational Health and Safety (NIOSH) official website. Key terms and phrases that were utilized were:

1. Occupational exposure and pesticides and heart disease
2. Occupational exposure, pesticides and stroke
3. Occupational exposure, pesticides and cardiovascular disease
4. Agricultural work and occupational exposure and fungicides
5. Agricultural work and occupational exposure and insecticides
6. Agricultural work and occupational exposure and herbicides
7. Industrial workers and occupational exposure and fungicides
8. Industrial worker and occupational exposure to insecticides
9. Industrial workers and occupational exposure of herbicides
10. Pesticide Applicator and cardiovascular disease
11. Pesticide Applicator and coronary heart disease
12. Pesticide Applicator and cerebral vascular accident

When excluding the phrase ‘pesticide exposure’ and expanding the search to the respective classes of fungicide, insecticide, and herbicide the database search results were enhanced.

Searching for ‘exposure’ and a specific job type also enhanced the search criteria results, which were chosen due to the high prevalence of blue-collar workers being exposed on the job.

Exclusion criteria involved any paper not written in English, any paper involving pesticide exposure mixed with another chemical type (e.g. Pesticides and Biphenyl A (BPA, Pesticides and Warfarin), animal studies, children and developing fetuses, secondary exposure of

family members, pesticide exposure's effect on the respiratory system, and any paper with developing cancers, types of tumors, and reproductive disorders. Many papers that had relevant abstracts on cardiovascular disease and pesticide occupational exposure were not available in their entirety in English on PubMed. Additional exclusions included population studies of pesticide exposure that were not occupationally related and studies not within the United States.

Inclusion criteria for the literature review included any mortality studies from pesticide exposure (herbicide, fungicide, and insecticide), which listed heart disease or cardiovascular disease as the cause of death. These articles on mortality studies mostly involved neurological diseases and different types of cancer, mechanical accidents, and circulatory diseases. Articles read when compiling information dated from 1975 to 2016.

Results:

Table 1: Pesticide Mortality and Morbidity for Circulatory Diseases Within The United States of America

Author	Article Title	United States Occupation	Pesticide exposure	Cardiovascular issues
Beaumont	Mortality in Agricultural Workers After Compensation Claims For Respiratory Disease Pesticide Illness and Injury	Agricultural workers	Pesticides	CHD mortality elevated among workers with systemic pesticide illness
Cantor	Mortality Among Aerial Pesticide Applicators and Flight Instructors	Pesticide applicators and flight instructors	Pesticides	Below expectation rate of arteriosclerotic heart disease
Cantor	Mortality Among Aerial Pesticide Applicators and Flight Instructors: A Follow-Up From 1965-1988	Aerial Pesticide Applicator	Pesticide	CVA mortality significant but not CHD mortality
Charles, Luenda	Occupational Exposure to Pesticides, Metals, and Solvents: The Impact of Mortality Rates in the Honolulu Heart Program; Occupational Exposure and Mortality	Multiple occupations	Pesticide	Mortality due to Circulatory diseases (CHD and CVA)
Das, Replay	Pesticide-Related Illness Among Migrant Farm Workers in the United	Farm workers	Pesticide	CHD illnesses undercounted for but not CVD mortalities

	States - California Pesticide-Related Illness Surveillance System			
Dayton	Pesticide Use and Myocardial Infraction Incidence Among Farm Women in the Agricultural Health Study	Farmers	Insecticide s, fungicides, herbicides	No significant myocardial infraction mortality in women although 6 of the 27 pesticides significantly associated with non- fatal my cordial infractions
Fleming, LA	National Health Interview Survey Mortality Among U.S. Farmers and Pesticide Applicators	Pesticide applicators, farmers Vs. non- exposed other occupation s	Pesticides	CVD/CHD mortality not significant compared to the unexposed population
Fleming, LA	Mortality in a Cohort of Licensed Pesticide Applicators in Florida	Pesticide applicators	Pesticides	No increase of CVD mortality compared to the general population due to the healthy workers effect for both males and females
Garcia, SJ	Methyl Parathion: A Review of Health Effects	Pesticide applicators	Methyl parathion	Leads to cardiac arrest
Kross, BC	Proportionate Mortality Study of Gulf Course Superintendents	Golf course superintend ent	Pesticides	Significant mortality due to arteriosclerotic heart disease
Maroni, M	Heath Effects in Man from Long-term Exposure to Pesticides: A Review	Farmers	Pesticides	Non-significant CVD due to the healthy worker

	of the 1975-1991			effect
Mills, KT	Pesticides and Myocardial Infraction Incidence and Mortality Among Male Pesticide Applicators in the Agricultural Health Study	Agricultural workers	Pesticides	No increase of morality from myocardial infractions or nonfatal myocardial infractions
Morton, W.E.	Hypertension in Oregon Pesticide-Formulating Workers	Pesticide manufacturing workers	chlorophenoxy & phenoxy	An increase in hypertension but due to family history
O'Malley, MA	Subacute Poisoning with Phosalone, an Organophosphate	Migrant farm workers	Phosalone	Significant cases of bardycardia
Schreinemachers	Mortality form Ischemic Heart Disease and Diabetes Meltus (type 2) in Four U.S. Wheat Producing States: A hypothesis Generating Study	Wheat agricultural workers	Chlorophenoxy herbicides	Ischemic Heart Disease increased for both men and women compared to the state's general population
Stubbs, Harrison	A Proportionate Mortality Analysis of California Agricultural Workers 1978-1979	Agricultural workers	Pesticides	CHD deaths lower than expected for both males and females
Sweeney	Human Health Effects After Exposure to 2,3,7,8 - TCDD	Chemical workers	2,3,7,8-TCDD	Significant CHD mortality
Sweeney	Human Health Effects After Exposure to 2,3,7,8 - TCDD	TCP workers	Herbicides	2x the rate of cerebrovascular disease but not CVD mortality
Sweeney	Human Health Effects After Exposure to	Ranch hand	2,3,7,8-TCDD	No association circulatory diseases

	2,3,7,8 - TCDD			
Rinsky , Jessica	Agricultural exposures and stroke mortality in the Agricultural Health Study	Farmers	Pesticides	Significant stroke mortality but due to mold (aphlotoxin) exposure instead of pesticides
Rodriguez & Prado	CDC - Acute Pesticide Related Illness Resulting of Occupational Exposure to Acrolein - Washington and California	Licensed aquatic pesticide applicators and pesticide equipment maintenanc e workers	Acrolein	inflammation of heart and ventricular fibulation in case studies continued years after exposure
Wang, HH	Mortality of Pesticide Applicators	Pesticide applicators	Pesticides	Deaths from cerebrovascular disease less than expected, thus not significant
Waggoner	Mortality in the Agricultural Health Stud: 1993-2007	Farmers, pesticide applicators (commerci al and private)	Pesticides	No increase in mortality from CHD or all circulatory disease compared to Iowa and north Carolina's state's population
Whiting William	Cardiovascular Disease & the Work Environment: A Critical Review of the Epidemiologic Literature on Chemical Factories	Chemical factory workers	Dioxin	Not significant increase in CHD

The majority of the articles that covered the topic of pesticide exposure and heart disease, stroke, or cardiovascular disease were mortality studies that relied on self-report of exposure, death records, company health records, or records from workman's compensation and insurance companies. There are no data on proper protective equipment (PPE), which was not mentioned in these studies. If proper protective equipment was worn and put on correctly, diseases from acute and chronic exposure rates could at least be minimized or possibly prevented. In a number of these papers, there was no definition for acute and chronic exposure to various pesticides. Among factory workers, some had acute poisoning and chronic poisoning in the same investigation, depending on the type of work within the same factory (13,14).

The majority of jobs from occupational pesticide exposure were jobs that involved manual labor, which were assessed mostly between the 1970s to the 1990s. Blue-collar workers are defined as laborers in occupations such as farming, ranching, pesticide application, florists, factory workers, orchard workers, construction, and industrial workers. Four studies have agriculture workers that include both farm and ranch tasks, harvesting, and pesticide application. There were seven pesticide applicator studies, of which three studies included combined either pesticide applicators with farm workers or flight instructors. Four studies analyzed pesticide occupational exposure of pesticide manufactory factory workers. Lastly there was one study that listed multiple occupations and one study that assessed mortality of golf course superintendents.

The majority of the studies observed pesticide exposures (fifteen studies) concerning mortality and cardiovascular diseases, and may include a mixture of pesticides. For example as a florist or pesticide applicator, you can be exposed to multiple types of pesticides over time. Seasonal agricultural workers depending on the crop are exposed to different pesticides throughout the year. One paper discusses phosalone (organophosphate), 1 listed herbicides, 1

study Acrolein (fungicide), and 1 assessed Methyl Parathion. Three discussed dioxins, which include TCDD and chlorophenoxy.

Most of the studies assessing occupational exposure to pesticides involved men. The two reasons for the overwhelming representation of men in these studies are that: 1. Most clinical trials are performed on men, and 2. In the past, men made up the majority of the work force and still hold most of the job positions in blue collar occupations. There were only three studies that included women, and one of these studies excluded men entirely (Dayton, Fleming, Schreinemachers). Of the 23 articles reviewed, the occupations were farm workers, agricultural workers, pesticide applicators and pesticide manufactory workers.

The majority of the literature reviewed concluded that, while assessing pesticide exposure, it is important to have a specific pesticide's name, regardless of what pesticide class it belongs to. Seven studies came to the conclusion of significant increase in CVD mortality or CVD diseases. Eleven studies had a significant decrease in instances of CVD mortality and circulatory diseases. Four studies of pesticide occupational exposure had mixed results for CVD related diseases and mortality. The discussion in this paper will assess the factors that produce differences in exposure, and will seek to explain why some papers stated no correlation to circulatory diseases and mortality, while other studies found conclusive evidence determining pesticide occupational exposure leading to circulatory diseases. Some studies had mixed results depending on the type of circulatory disease and CVD mortality.

Discussion

Adverse effects of exposure to chemicals involve many variables and complex biochemical pathways that have not been fully articulated or understood. The route of exposure has to be assessed to determine how the body will process and break down the chemicals, but this is also dependent on the duration of exposure. In these studies there exists a different definition of what is considered long-term exposure. In some studies, long-term exposure is considered the cutoff limit to find significant results. In some cases, long-term exposure of more than 15 years in the Kuakini Honolulu Heart Program showed a positive correlation with heart disease mortality (15). One study determined that long-term occupational exposure means five or more years (16). According to Leiken (17), exposure to pesticides can happen during the manufacturing process and the application process, usually occurring through inhalation and dermal absorption.

In addition to routes of exposure, other factors play a significant role in the relationship between exposure and disease, which include gender, age, genetics, lifestyle, environmental factors, the efficiency of individual metabolization rates to expel chemicals, and nutrition. In Iowa farm country analyzed pesticide exposure between spouses and family members the same task involving the use of pesticides(18), the wives and female pesticide applicators had an increased risk of cardiovascular issues, but the men in the same cohort did not, suggesting there must be some biochemical pathway or hormone-related factor that makes men and women respond differently to these chemicals while employed in the same type of labor. A study of POPs, which found an association with high blood pressure, found that different types of pesticides cause hypertension in one sex but not the other (19). NHANES data concluded that exposure to PCDD and PCDFs, which was measured via collecting serum, caused hypertension

in women, yet PCBs caused hypertension only in men. In contrast, organochlorines were not found to cause high blood pressure in either gender (20). Van Larebeke (21) found similar results for pesticide exposure in males vs. females, not only for hypertension but for diabetes too. Biomarkers for exposure are still being established for chemical occupational exposure.

In addition, when breaking down these chemicals, sometimes the metabolite is more toxic than the parent compound. Some of these chemicals can have more than one metabolite. For example, DDT breaks down into DDE and DDD. Some fungicides like carbenzium are more toxic when broken down into their metabolites and have since been taken off the market. Another aspect to consider when dealing with long-term chemical metabolism, is that some pesticides are fat-soluble and others are water-soluble, yet both have a long half-life. The Vena, et al. study of factory workers and pesticide applicators found the effects of exposure from 20 years prior still persistent in the body. Vietnam veterans exposed to Agent Orange still had persistent chemicals in their body contributing to diseases such as diabetes, hypertension and heart diseases. The following paragraphs discuss information pertaining to diabetes, pesticide chemical class exposure and cardiovascular diseases.

Organophosphate

One study of 20 individuals exposed to high organophosphate led to an abnormal electrocardiogram (EKG) analysis associated with ventricular arrhythmias, tachycardia, and bradycardia with attributes of myocardial ischemia (22). Organophosphates and Carbamates have been linked to cholinesterase inhibition. This is a crucial finding as build-up of cholinesterase can lead to parasympathetic effects of the cardiovascular system (23). Mixed

pesticides of organophosphates, carbamates, organochlorine & pyrethrine lead to atherosclerotic changes in the vascular wall, resulting in changes in systolic and diastolic blood pressure in male mosquito control sprayers (24).

Nitrate Pesticides

Organic Nitrates lead to vasoconstriction of the coronary arteries that causes heart disease, hypertension, & hypotension (25). According to Kristensen (26), there is a strong relationship between heart disease and aliphatic nitrates in relation to CVD studies, which has been noted in studies since at least the 1950s. Exposure and then withdrawal symptoms to nitroglycerin and ethylene glycol dinitrate have caused sudden heart attacks, arrhythmia and sudden death.

Dioxins

Exposure to the class of herbicides called dioxins was discussed often in mortality studies of pesticide exposure. Workers in the 1930s up to 1958 who worked in weed control, sprayed herbicides in the hot summer months. They would often spray the pesticide on themselves to cool off, which resulted in developing higher rates of heart disease than the average population (27). Dutch factory workers' exposure to 2,4,5 – TCDD showed no evidence of an increased risk of developing cardiovascular diseases.

The Vena et al study reported significant diseases (28) (including circulatory diseases) in relation to the neighborhood vicinity from the hot zone of the accidental factory spill. Dioxins such as Agent Orange and Agent Purple were widely used during the Vietnam War. One study focused on veterans, although the level of exposure to dioxin is unknown. No relationship was identified with the circulatory system, except for acute myocardial infarction and atherosclerosis

(29,30). Cardiovascular disease was increased, as was angina, ischemic heart disease, and stroke, but not myocardial infarction or hypertension. The results from this study were inconclusive as statistical significance was present in some cardiovascular diseases but not others. Diabetes increased and displayed a positive correlation in this study. Dioxins such as TCDD have been associated with hypertension and diabetes (31,32). Mortality from cardiovascular diseases also appeared to be elevated among cohorts of exposed chemical workers and Seveso residents in Italy (33). Continued surveillance of the health of exposed populations will be useful in identifying the long-term effects of both high and low 2,3,7,8-TCDD exposure, thus establishing biomarkers.

In addition, Sweeney looked at TCDD exposure in various occupations in the United States and Europe. The United States did not report higher rates of cardiovascular mortality from TCDD exposure but certain European countries did. Other herbicide studies have reported no association with heart disease, stroke, or cardiovascular disease, which could be due to the healthy worker effect (34).

Studies found that the lack of association between pesticide exposures with cardiovascular diseases could be due to the healthy worker effect. Studies in Florida pesticide applicators and California farmworkers were also healthy workers, that may explain the reason why pesticide exposure was not associated with cardiovascular diseases. The workers had to be healthy to conduct the physically strenuous agricultural work (35,36). The healthy worker effect is defined as a phenomenon observed initially in studies of occupational diseases; workers usually exhibit lower overall death rates than the general population because severely ill and disabled people are excluded from employment. These studies stated that farm families tend to have healthier and more active lifestyles than the general population. Another reason why there

may be no association with cardiovascular diseases is due to agricultural and farm workers having the highest mortality rates for mechanical or vehicle accidents and cancer compared to any other occupation (37,38). A study entitled “Mortality and Agricultural Workers” found lower mortality rates among farmers and pesticide applicator workers in North Carolina and Iowa compared to the general populations in men. In the same study it was noted that pesticide applicators had an increase in cardiomyopathy but it wasn’t statistically significant (39). This finding could be because agricultural workers get more physical exercise than the average American during their working hours and have a high rate of dying from mechanical accidents and cancer before heart disease develops.

In Californian agricultural studies, pesticide exposure is listed under accident types and injury related work. ‘Cardiovascular Strain’ due to unknown causes on the job is classified under fatalities according to workman’s compensation guidelines, although technically it is an occupational exposure, which could be fatal in some cases. In the 1970s, Workman’s compensation suggested that there were increased filings for workman’s compensation but not necessarily due to pesticide exposure. They suggested that pesticide-related injuries were underreported in Californian agricultural studies because during chronic low-dose exposure, the human system mimics that of either heat stroke or excessive alcohol consumption (40). Data for the study were only collected for one month and illegal immigrants tend to not report occupational injuries, further limiting the number of reported pesticide-related injuries.

Occupational pesticide exposure is also under-reported due to a lack of recognition of the signs and symptoms of exposure and no access to healthcare for migrant agricultural workers. Thus occupational pesticide exposure is underreported.

In case studies of migrant workers, most immigrant workers were exposed to a mixture of pesticides. In the 1980s, exposure to a mixture of phosalone pesticides that caused increase heart palpitation and chest pains that were then elevated when the pesticide was metabolized, although current OSHA and EPA regulations were implemented. This multiple case study led to a change in regulation, requiring waiting a week after application of spray of pesticides before harvest in California. The physical and physiological demands of on-the-job stress and pesticide exposure over time lead to adverse health outcomes like cardiovascular diseases, which progress slowly in immigrant farm workers (41).

According to the CDC, most of the acute cases were case reports of one individual who had experienced acute pesticide exposure from accidental occupational poisoning and their severe symptoms from pesticide drift included bradycardia and abnormal EKGs, and non-fatal myocardial infarction. Doctors need to be trained to recognize the signs of pesticide exposure, especially in agricultural areas.

Health statistics of migrant farm workers in America are hard to keep track of due to traveling work from crop to crop, the prevalence of illegal immigrants in these roles, inadequate health care, and not taking time off work to go to the doctor. It has been noted that migrant and seasonal workers suffer from hypertension, diabetes, obesity, and depression and are exposed to a variety of mixed pesticides on the job (42). According to McCauley, half of them are seasonal workers and receive their healthcare in Mexico during the off-season. When they do have symptoms of heart disease and it is not harvest time, the death certificate lists a different occupation or none at all. According to Bohme,(43) farm workers are not required by OSHA regulation under the “Right to Know Act” to know what pesticides they are being exposed to while only the pesticide applicator is required to have proper training. Arcury notes that farm

workers are exposed to many different pesticides, such as organophosphates and carbamate and pyrethroid insecticides and several herbicides. Farm workers and immigrant workers are prone to depression, which is thought to be due to pesticide exposure. The biomarker that is associated with depression is cholinesterase (44). This biomarker's measurement is important because it is the same biomarker that, when exposed to pesticides in the past, correlates to heart disease and cardiovascular diseases. Screening for cholinesterase inhibition from organophosphate exposure is now mandatory in California for pesticide handlers and applicators (45). Hypertension is another of the health related issues of immigrant farm workers (46).

Conclusion:

Pesticide exposure was analyzed in relation to CHD, CVD, and CVA for mortality. The majority of the literature provided inconclusive results and mixed reviews. This literature review suggests occupational pesticide exposure in relation to disease development is dependent on many factors. Such factors include the chemical class, duration and dosage of occupational chemical exposure combined with risk factors such as gender, age, and life style choices. Due to the nature of the occupations analyzed, most of the population was male. Of the three types of circulatory diseases, the least amount of information could be found on stroke. Some studies have a positive association with cardiovascular diseases and still others proved to be inconclusive regarding heart disease, stroke, and cardiovascular disease. Due to the complex nature of the cardiovascular system, damage mirrored by other symptoms, high cancer occurrence among occupations and pesticide use, the direct correlation and complex relationship between the two still needs to be further assessed. Further research is needed to determine the role of pesticides in the development of cardiovascular disease incidence.

Chapter Three: Occupational Exposure of Pesticides is associated with the Development of Cardiovascular Diseases

Introduction:

According to the World Health Organization (1) cardiovascular diseases were the number one cause of death worldwide, and account for 31% of all deaths annually. The purpose of this study is to determine if there is an association between occupational exposure to pesticides and the incidence of cardiovascular diseases (CVD), including coronary heart disease (CHD) and stroke (CVA). Most scientific articles examining chemical occupational exposure and stroke (CVA), coronary heart disease (CHD) and cardiovascular disease (CVD) look at mortality only (2,3). The present hypothesis is that occupational exposure to pesticides plays a synergistic role in developing CHD, CVA and cardiovascular disease (CVD) in men of Japanese descent, however, the mechanisms and biochemical pathways by which pesticides cause the development of these circulatory diseases are not yet fully understood.

The intention of the Kuakini Honolulu Heart Program (HHP) was to study the incidence of cardiovascular diseases, including the different types of stroke, and heart disease, and to determine the risk factors associated with these illnesses. Later the same cohort from Kuakini HHP was used to study neurological diseases in the elderly population and this study was called

the Kuakini Honolulu - Asia Aging Study (HAAS). From these two studies of the same cohort, papers on occupational exposure to pesticides, solvents and metals in relation to total mortality, cancer, Parkinson's disease, bone fractures, Alzheimer's and dementia have been published (10,2,4). Occupational exposure was assessed at the first examination for types of occupations and duration of occupation over the person's lifetime. These jobs were categorized according to the U.S. Bureau of the Census at the time. The first examination took place between 1965-1968 and these are the data we are using for our baseline exam.

There have been multiple occupational studies of this cohort associated with various diseases such as cancer, Alzheimer's disease, and Parkinson's disease, however, cardiovascular disease incidence has not been looked at yet. One study that was particularly interesting was the assessment of occupational exposure of chemicals in relation to total mortality from circulatory diseases, respiratory diseases, and cancer. Mortality from causes of death were 28.3% for circulatory disease, cancer was 32.4%, respiratory disease was 8%, diabetes, digestive disorders, and other disease were put into one category totaling 13.1%, accidental deaths was 13.1%, and undetermined causes was 14.4%. Respiratory diseases and all types of cancer were associated with all three occupational chemical types. Circulatory disease was only associated with solvents and pesticides. The mortality analyses were broken down into various lag times, every five years. The fifteen year lag time was found to be the most relevant in relation to circulatory diseases mortality from solvents and pesticides. Exposure of chemicals was divided into range scores of none, low, medium, and high which will be further discussed in materials and methods section. According to Nelson, the metals they were exposed to were manganese, iron, and mercury. Solvent exposure predominately was carbon tetrachloride and carbon disulfide. Pesticides were identified as insecticides, fungicides, and herbicides but not specifically

named (4). All these chemicals have been reported to cause cancer and that is the most common disease resulting from occupational exposure of chemicals. Heavy metals and carbon disulfide have been associated to cardiovascular diseases (5,6). The Parkinson disease paper from the Kuakini Honolulu Heart Program by Petrovitch stated that 68.8% of the study cohort worked sometime in their lifetime in the plantations, exposing over 6,000 individuals to chemical exposure of pesticides and also of solvents, dust, metals, and polycyclic aromatic hydrocarbons (gasoline, diesel).

The current study builds on these papers and examines the relationship between chemical exposure and CVD, CHD and CVA incidence. Most papers concerning occupational exposure to pesticides examine mortality, neurological disorders, and cancer. In this study, the hypothesis is that occupational exposure to pesticides plays a role in the etiology of cardiovascular disease (CVD) in men of Japanese ancestry, although the mechanisms and biochemical pathways are not entirely understood. The data were collected from middle-aged Japanese-American men that participated in the Kuakini Honolulu Heart Program (HHP). Prevalent cases of CVD, CHD and Stroke at baseline will be excluded. The Occupational Health and Safety (OSHA) scale for permissible exposure limits (PEL) in relation to chemical exposure and duration of primary and current jobs will be used to assess intensity level of exposure for the Kuakini HHP cohort, which is the same OSHA scale previously used in Kuakini HHP and HAAS. The findings of this study hope to identify the possible role of pesticides in the development of cardiovascular diseases in order to prevent related CVDs in the future.

Pesticides of various types have been associated with CVD in other studies at high exposure rates, especially in subjects who would wear little or no proper protective equipment (PPE). For example, factory workers involved with Phenoxy Herbicide and Chlorphenol

Production, were found to have an increased incidence of circulatory disease, including ischemic heart disease and diabetes (Vena, 1998). According to the Hawaiian Department of Agricultural in 1969, common pesticides in Hawaii consisted of various classes of organophosphates, organochlorines, and herbicides. Although pesticides were used as chemical warfare agents during World War I and World War II, they were not used commercially until 1945 (8). Most of these chemicals have since been banned, such as chlordane, DDT, dieldrin, heptachlor, hexachlorobenzene, toxaphene, as they are persistent organic pollutants. Pesticides, depending on their type of solubility, go through degradation into different metabolites, but they never go away. The jobs that have been associated with pesticide exposure and possible cardiovascular disease are either agricultural or industrial. In general, occupations that are associated with pesticide exposure are: pesticide applicators, craftsman, landscapers, forestry, factory workers, pesticide manufactures, aircraft mechanics, jet fuel refineries, agricultural work.

Materials and Methods:

Study Sample:

Data from the Kuakini HHP cohort were collected over a thirty-four year period from the mid-60s to the late 1990s in Japanese-American men on the island of Oahu in Hawaii. The Kuakini Honolulu Heart Program enrolled 8,006 participants out of 11,000 possible candidates from a listing of World War II Selective Service records (Worth). These men were recruited via mail and phone calls. Participants were born between 1900 – 1919 either in Japan or Hawaii. Therefore, this population consisted of immigrants or second generation Japanese Americans between the ages of 45 to 68 (Charles, Petrovitch, Kagan). Occupational data that was collected

at baseline exam is only available for 7,994 individuals. All cases of CVA, CHD, and CVD were removed at baseline before statistical analysis leaving the total sample size at 7, 557 participants.

Occupational Exposure Analysis:

An OSHA Scale was created and coded by industrial hygienists at the National Institute of Occupational Safety and Health (NIOSH) based on permissible exposure limits (PEL) for each occupation (Charles, 2016). PELs determine the maximum amount of chemical exposure a person can be exposed to over a time-weighted average (TWA). The TWA is the average amount of exposure over a specific period, mainly eight hour work day or a forty hour work week. This OSHA code was developed to analyze data for various papers on occupational exposure in the Kuakini Honolulu Heart Program by creating intensity scores for metals, pesticides, and solvents served as independent variables as described Charles and were based on Industry-occupation exposure variables, time variables (years worked and individual's age during that job), and agricultural exposure variable (Kasson). They had four categories, which were none (0), low (1), medium (2) & high (3). The ranges of zero corresponded to no exposure, low exposure was the range from 1-39, medium 40-79, and high was 80 and above. The OSHA exposures scale levels are further broken down into range scores of exposure for categorization (Charles, 2016). Occupational exposure collected at baseline and the third examination were used for the analyses. Participants were asked about their past and present jobs at the time, the ages they started working and finished working in these occupations (Charles). The majority of the jobs (66%) involved manual labor (craftsmen, farmers, laborers, crane operators, & service workers (Charles, 2010). Professional occupations (chemist, agricultural scientist, chemical engineer) only included 7.8%, 9% were clerks, 7.6% were managers, 7.3% were salesman, & 2% were technicians (Charles, 2010). According to Kashon and Burchfiel three parameters were taken

into account when identifying exposure to pesticides, which were: 1. If exposure of any kind occurred at any time, 2. An estimate of the magnitude/duration of the exposure, 3. The particular timing of the exposure. In conclusion, by using these criteria mentioned above the industrial hygienists were very confident about the range scores that were assigned to create each intensity level of exposure for the chemical classes.

Statistical Methods:

Prevalent cases of cardiovascular disease were excluded from the original data analysis when the study first began. The original sample size was 8,006 male participants, however after excluding cardiovascular disease at baseline and those with missing data for occupation, the final sample size for analysis was N=7557 Japanese American Men. The mean values and standard deviation of the covariates at baseline for the study population were calculated. The Cox proportional hazard models for each outcome (incidence for CHD, stroke, and CVD) for the various levels of pesticide exposure relative to no exposure and various risk factors, including age, SBP, smoking, cholesterol, triglycerides, physical activity, alcohol intake, glucose, & education were calculated. Analysis of variance models were used to obtain the mean values of covariates across levels of pesticide exposure. The analysis of variance models were used to obtain the mean values of covariates across levels of pesticide exposure. Univariate methods were used to compare baseline characteristics of patients who had CVD. The Cox proportional hazards models were run for the first ten years (1965 to 1974), second ten years (1975-1984), & then the final 14 years (1985-1999), as well as the overall period of 34 years.

The dependent variable in this study is cardiovascular disease (CVD) incidence.

Baseline co-variables were: age (yrs.), systolic blood pressure or SBP (mmHg), diastolic blood pressure or DBP (mmHg), total cholesterol (mg/dL) triglyceride (mg/dl), glucose (mg/dl), BMI, physical activity index, smoking (pack-years), Alcohol (oz/month), education (% that graduated from high school) Incidence rates of CVA, CVD, CHD were evaluated per thousand person years follow-up, and adjusted for age.

In addition, Cox Proportional hazard models were analyzed for each exposure level (none, low –moderate combined, and high) for pesticides and adjusted for the various risk factors that were established at baseline. Low and medium exposure level were combined to make a new category of low-moderate because the sample size for pesticides was small in the medium level of exposure. The analyses were conducted initially without risk factor adjustment, adjusting for age only, adjusting for all risk factors except BMI, adjusting for all risk factors except SBP and all risk factors included in the analyses. The lag times used for event occurrence were for the first ten years, second ten years and the last fourteen years. The purpose of taking out SBP, and BMI from the model was to see if there was any effects mediated by these known risk factors. All of the statistical analysis was conducted using the software Statistical Analysis Systems (SAS). Analyses were also conducted for CHD and CVA separately, in addition to CVD but the results for CHD and CVD were not significant, as the incident numbers were small. In addition, analyses were also conducted for other exposures of interest, including metals and solvents, but these results were not significant either. Therefore these analyses are not shown in this manuscript.

Results:

Table 2 : Baseline Cardiovascular Disease Risk factors by Disease Status (Incident CVD)

variables	NO CVD n= 5,028	CVD n = 2,549	p-value
Age, years	54.20 ± 5.52	54.59 ± 5.60	0.0038
BMI, kg/m ²	23.55 ± 3.11	24.36 ± 3.04	<.0001
SBP, mm Hg	131.02 ± 19.73	139.10 ± 21.68	<.0001
DBP, mm Hg	80.74 ± 11.18	84.85 ± 11.89	<.0001
Total cholesterol, mg/dL	214.91 ± 36.83	223.52 ± 39.48	<.0001
Triglyceride, mg/dL	223.79 ± 188.54	260.81 ± 228.18	<.0001
Glucose, mg/dL	157.20 ± 54.26	168.50 ± 64.01	<.0001
Physical Activity Index	32.96 ± 4.56	32.79 ± 4.49	0.1389
Smoking, pack-years	23.30 ± 24.43	24.63 ± 24.40	0.0269
Alcohol, oz./month	14.77 ± 25.15	12.65 ± 23.49	0.0004
Education, high school %	48.87 ± 49.99	49.84 ± 50.01	0.422

Table 3: Unadjusted Baseline CVD Risk Factors by Levels of Pesticide Exposure

Risk Factors	No Exposure to Pesticides n =7,016	Low-moderate exposure n = 110	Pesticide High exposure n =451	p-value
Age, years	54.18 ± 5.49	55.14 ± 5.46	56.61 ± 5.99	<.0001
Body Mass Index, kg/m ²	23.83 ± 3.12	24.10 ± 3.11	23.63 ± 2.97	0.1717
Systolic Blood Pressure, mm Hg	133.67 ± 20.62	137.09 ± 22.62	133.90 ± 22.36	0.8243
Diastolic Blood Pressure, mm Hg	82.18 ± 11.54	83.27 ± 12.45	80.88 ± 12.04	0.0202
Total Cholesterol, mg/dL	217.78 ± 38.00	215.34 ± 44.60	218.81 ± 35.46	0.5799
Triglyceride, mg/dL	238.18 ± 204.21	219.86 ± 163.01	210.69 ± 199.86	0.0063
Glucose, mg/dL	160.80 ± 58.08	169.50 ± 55.21	162.15 ± 56.69	0.6308
Physical Activity Index	32.80 ± 4.52	33.48 ± 4.53	34.38 ± 4.60	<.0001
Smoking, pack-years	23.85 ± 24.51	25.33 ± 24.24	21.86 ± 23.22	0.0962
Alcohol, oz./month	14.35 ± 24.82	12.44 ± 20.43	9.83 ± 21.96	0.0002
Education, high school %	50.53 ± 50.00	38.18 ± 48.81	31.04 ± 46.32	<.0001

Values are means (± SD) or percentages. [1] Pack-years were calculated only for current and past smokers

Table 4: Incidence Rates of Cardiovascular Disease by Pesticide Groups

Incidence Rates for CVD for the first ten years				
Exposure	sample size	CVD	unadjusted	age adjusted
none	7016	550	8.34	8.48
low moderate	110	5	4.74	4.28
high	451	51	12.2	10.74
Incidence Rates for CVD for the last thirty four years				
Exposure	sample size	CVD	unadjusted	age adjusted
none	7016	2356	14.46	14.83
low moderate	110	32	12.93	12.61
high	451	161	16.04	15.28

*CVD rates per 1,000 person/per-years follow-up

Table 5: Cox Proportional Hazard Ratios of No Exposure vs. Low -Moderate and High Exposure to Pesticides based on Intensity Scores during the First Ten Years of Follow-up

Variables	Low-Moderate	p-value	high	p-value	P for trend
Unadjusted	0.57 (0.24-1.37)	0.209	1.46 (1.10-1.95)	0.009	0.023
Age	0.54 (0.22-1.30)	0.166	1.27 (0.95-1.69)	0.108	0.202
Risk factors no BMI or SBP	0.54 (0.23-1.31)	0.176	1.40 (1.04-1.89)	0.027	0.064
Risk factors + SBP only	0.49 (0.20-1.19)	0.116	1.42 (1.05-1.91)	0.022	0.065
Risk factors + BMI only	0.53 (0.22-1.29)	0.163	1.40 (1.04-1.89)	0.028	0.067
All Risk Factors	0.49 (0.20-1.19)	0.116	1.42 (1.05-1.92)	0.021	0.062

Figure 1: Kaplan-Meier Curve of Cardiovasclar Incidences

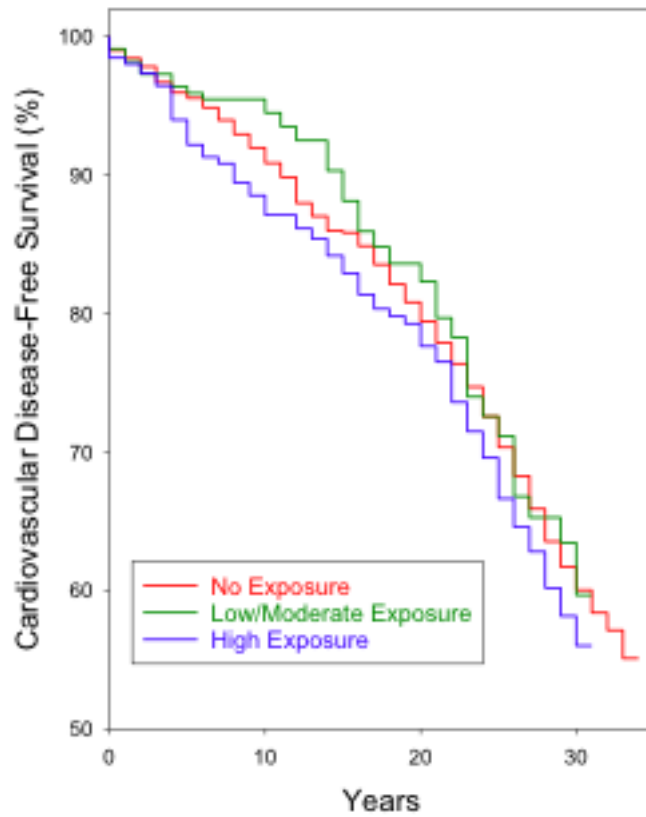


Table 2. Baseline Participant Characteristics by Incident Cardiovascular Disease Status

This table displays descriptive characteristics of participants at the baseline exam,

comparing those with and without incident cardiovascular disease. As expected men who developed cardiovascular disease during follow-up had a worse risk factor profile at baseline than those who did not. Those who developed incident cardiovascular disease were slightly older, had higher BMI, higher blood pressure, higher cholesterol, triglycerides, glucose, smoked more and consumed less alcohol compared to those with no CVD ($p<.05$). There were no differences for physical activity index or education level.

Table 3. Baseline CVD Risk Factors by Pesticide Exposure Groups

Table 3 gives descriptive characteristics of CVD risk factors based on the intensity levels of exposure. Occupational exposure to pesticides was inversely associated with diastolic blood pressure, triglycerides, alcohol, and education; and also directly associated with age, physical activity ($p<0.05$). No relationship was observed for body mass index, systolic blood pressure, cholesterol, glucose or smoking.

The healthy worker effect with higher levels of physical activity would explain why the group with high exposure to pesticides also had lower diastolic blood pressure and triglycerides. Low –moderate exposure group has the have the highest risk factors for developing cardiovascular disease,, but the smallest sample size. No exposure group consumes the most alcohol and had the highest education level. There is some social economic status between both alcohol consumption and level of education between the groups of participants because of the huge number contrast between groups.

Table 4. Incidence Rates of Cardiovascular Diseases by pesticide exposure groups

This table displays unadjusted and age adjusted incidence rates per 1000 person-years follow-up by levels of exposure (none, low-moderate, and high) for the first ten years of follow-up and for total follow-up period of thirty-four years. The number of subjects in each group is shown. Trend tests were non-significant, although more people develop CVD in the highest exposure group.

Table 5. Relative Risk for Low-Moderate and High Exposure to Pesticides versus No Exposure, based on Intensity Scores During the First Ten Years of Follow-up

The purpose of this analysis is to assess the relative risk of different exposure levels compared to no exposure. In these analyses we found that CVD incidence was not associated with low to moderate levels of exposure to pesticides, in unadjusted and adjusted models. Although low moderate exposure is not significant it appears to be protective. This thought to be due to the Hermonesis Principle as in low doses can be protective and stimulate homeostasis of the organism, yet sensativity varies from individual to individual. However, high exposure to pesticides was significantly associated with CVD incidence both in unadjusted models and various models adjusting for risk factors, except when only adjusting for age. SBP and BMI were removed from adjustment models at separate analysis to determine if they could mediate these relationship but no confounding effects were found. The adjusted models included the risk factors of age, total cholesterol, triglycerides, glucose, physical activity, smoking, alcohol and

higher education, BMI and SBP. The lag times of the second ten years of follow-up and overall thirty-four years of follow-up were looked at and were not found to be statistically significant. In summary, cardiovascular disease incidence for the ten year follow-up was found to be associated with high-intensity scores of pesticide exposure when compared to no exposure in unadjusted and adjusted models.

Graph 1. Kaplan-Meier Curve

Kaplan – Meier curves estimates disease-free survival in the time to event or to the development of cardiovascular diseases over the overall follow-up period of thirty-four years, in relation to pesticide exposure of various levels of intensity. This graph shows that the group with higher exposure to pesticide had a higher incidence of cardiovascular diseases in the first ten years. It is represented by the lowest curve. The middle curve corresponds to no exposure of pesticide exposure and has the most individuals in that group. The highest curve develops cardiovascular disease at the slowest rate over the thirty-four year period and is the low-moderate exposure. Although they have the worst cardiovascular risk outcome from table two the pesticides seem to be protecting them from developing CVD a slower rate, which is called hormesis. The purpose of performing this type of analysis is to aid in identifying lag times for data analysis. Based on this curve, it appears that the curves separate the most at 10 years of follow-up. Based on these findings we conducted further analyses for this project using 10 years of follow-up instead of the 34 years, when the curves seem to converge again. This graph helped us determine the follow-up lag times to consider for statistical analysis.

Discussion:

In the Charles mortality paper of the Kuakini Honolulu Heart Program, circulatory diseases accounted for 28% of all deaths, and found that cancer was the number one cause of death, accounting for 32% of all deaths. In this research study, we examine the relationship between incidence of cardiovascular diseases and occupational exposure to pesticides.

In our research, a high level of pesticide exposure in the first ten years of follow-up was found to be statistically significantly associated with cardiovascular disease incidence without adjustment and after adjusting for all relevant risk factors. Vena et al. also found the highest susceptibility for developing circulatory diseases occurred ten to nineteen years after exposure to pesticides, in pesticide factory workers and sprayers of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD). Although we did not find any correlation at 17 years or 34 years after exposure, it is thought that other effects of aging may mask the effects of toxic pesticide exposure. According to Charles, the biggest correlation with chemical exposure (pesticides, metals and solvents) and total mortality was fifteen years prior to death. That study did not address incidence of CVD.

In our study, we also examined the relationship of occupational exposure to metals and solvents for cardiovascular disease incidence, but we did not find a significant association at the various lag times examined during the study. It is important to note that we also examined the outcomes separately for incident CHD and CVA and no relationship was found for either endpoint. Incident CVA showed an association with pesticide exposure at a p-value of 0.018, but was not significant in the adjusted models.

Perhaps participants in this study, especially those in manual labor type of jobs, could have exhibited a healthy worker effect due to higher levels of physical activity which may lower

the incidence of CVD overall and perhaps this may also attenuate the effect of pesticides on CVD incidence. Workers with low-moderate exposure to pesticides had a relatively small number of outcomes in the first ten years, since the group consisted of only 110 people.

Since the Occupational Safety and Health Administration (OSHA) wasn't established yet, proper protection equipment (PPE) wasn't required to be worn at the time the data were collected. Routes of pesticide exposure would have been absorption, inhalation, and ingestion. In addition, occupational exposure probably had a synergistic effect due to exposure to a combination of pesticide chemical classes together. Pesticide exposure has previously been linked to developing Alzheimer's disease, dementia, cancer, and Parkinson's disease in Kuakini Honolulu Heart Program studies (10). A study published in 2015, found an association between hypertension, pesticide exposure, and cognitive decline (14). According to Charles, Petrovitch in the Hawaii Agricultural Study, plantation employees were exposed to organochlorines, organophosphates and synthetic herbicides. Agricultural work would not be the only occupation in which pesticide exposure could occur, as scientists developing agricultural chemicals and technicians would also have on the job chemical exposure. Based on previous studies, pesticide exposures associated with the development of cardiovascular disease and coronary heart disease, have been attributed to organophosphates, organochlorines, and herbicides (14,15,16,17,18,19). Cardiovascular disease of this cohort has been looked at in relation to mortality and chemical exposure. It was important to adjust for other cardiovascular risk factors measured at baseline since pesticides have been linked to hypertension in previous studies. PCDDs and PCDFs were only found to cause hypertension in women, but not men. (23). PCBs and chemicals that act similar to dioxin cause hypertension in men (23). In addition, Diazoxanase used by mosquito sprayers were found to be associated with increased rates of

hypertension (18). Hypertension appears to be a strong risk factor for CHD, CVA and CVD, as well as mortality.

A previous study examining the first ten years of the Kuakini Honolulu Heart Program in relation to hypertension and mortality, found a link between both systolic and diastolic blood pressures with respect to developing CVD and subsequent risk of death within the first three examinations. The study also found that hypertension doubled the odds of death. The study found that systolic blood pressure (SBP) and diastolic blood pressure (DBP) were the most important independent risk factors for developing CVD and mortality (CHD, CVA, and CVD). Systolic blood pressure was strongly associated with an increase in the incidence rates of CHD and CVD. For stroke mortality, diastolic blood pressure was the most important predictor of stroke and was a stronger predictor than systolic blood pressure. Based on this article by Yuno, cancer was the number one cause of mortality at 36% and cardiovascular disease was the second most common cause of death at 34% in the first ten years of follow-up in this cohort. In our study while running unadjusted risk factors diastolic blood pressure was found to be a significant risk factor among all chemical types. After adjusting the model for age at baseline for pesticide exposure found diastolic blood pressure and systolic blood pressure statistically significant risk factors at baseline. In previous studies, pesticide exposure has had a direct correlation to an increase in hypertension in agricultural studies, which is a characteristic of CVD & other diseases (20,21,22,23).

According to Hung, an association between long -term effects of acute organophosphate poisoning was found in the development of CVD, arrhythmias (1.6 times higher), coronary artery disease, and congestive heart failure in the first ten years of exposure, in middle-aged men. This study confirms the evidence of the data from the Kuakini HHP cohort on

pesticide occupational exposure within the first ten years. In the Kuakini HHP study, we have additional risk factors that the Hung study didn't have access to in their cohort. Summarization of Hung's literature review stated that the underlying mechanism leading to CVD in younger people was the disruption in autonomic dysfunction due to the organophosphates effect on the neurotransmitter that controls cardiac muscles, inducing oxidative stress. The ACHE enzyme and its neurotransmitter aids in controlling the function of all muscles (skeletal, smooth, and cardiac). Pesticide interference with neurotransmitters is a reasonable explanation because many pesticides are neurotoxic. The Hung study also reported that the association of organophosphate exposure with CVD was masked by other risk factors as the Taiwan cohort aged.

In previous studies, biomarkers have been taken from blood serum, such as leukocytes, and adipose tissue, and lipoproteins (HDL, LDL, VLDL, and total cholesterol) to study pesticide exposure. Some of these biomarkers are also associated with CVD, CHD and diabetes (24, 25, 26). Organophosphates, organochlorines, and carbamates inhibit cholinesterase enzyme activity, which are responsible for breaking down neurotransmitters, such as Acetylcholinesterase (ACHE) and other cholinesterases. A decrease in ACHE is associated with hypertension in some agricultural studies, thus it has been suggested that enzyme activity of ACHE and BuCHE activity should be monitored as a risk factor for pesticide exposure (27). Organochlorines and carbamates have been shown to damage the central and peripheral nervous systems and smooth heart muscle (24).

TetraChloroDiabenzo-p- Dioxins (TCDD) exposure was still found in lipoprotein serum 20 years after high dosages of chronic exposure of farm workers in the Czech Republic of Eastern Europe. This exposure has been shown to play a role in atherosclerosis development and high blood pressure (28). High pesticide exposure affected cholesterol-making cells in the liver

leading to oxidative stress and hyperlipidemia, and finally to cardiovascular morbidity (29). The parent compounds and their metabolites resulting from chronic or acute pesticide poisoning still linger in the body decades after exposure, as some pesticides, such as TCDD, have a long half-life. In a separate study, Remnant-like particles (cholesterol and triglycerides versions) are also independent predictors of coronary heart disease in the Kuakini Honolulu Heart Program for cardiovascular risk factors, but not total triglycerides (29). With respect to lipoproteins, PCBs and organochlorines, exposures were found to be associated with an increase in developing CVD via affecting the production of high-density lipid (HDL) by reducing arylesterase activity as a function of pesticide concentration. PCB decreases the PON1 gene activity, which in turn impairs the HDL function, thus affecting cholesterol production (25). The PCBs also prevent the oxidization of low-density lipoproteins.

The Wafa study suggests that the inactivation of the PON1 gene causes the decrease in HDL production. Some of the functions of the PON1 gene include reducing oxidative stress, lipid metabolism and production of HDL, it reduces inflammation, and rids the body of pesticide toxicity (30,31, 32, 24). Genetic polymorphisms of the PON1 gene affect production of metabolic enzymes, especially ones that aid in cholesterol production and different neurotransmitters. The PON1 gene make enzymes related to both cholesterol production and for the breakdown of neurotransmitters. The ability of the PON1 gene to rid the body of pesticides and metals is polymorphism - dependent in the Turkish population (31,34). Some PON1 gene alleles are better than others at ridding the body of toxins, while other alleles make subjects more susceptible to developing coronary artery disease (CAD). Different populations carry different variants of the PON1, PON2, and PON3. The PON1 gene is associated with vascular diseases (35). According to Costa, the PON 1 gene aids HDL via high affinity reabsorption to

leave the liver via assistance from Apolipoproteins and phospholipids. A study conducted on the Kuakini HHP cohort found that Apolipoproteins predict CHD only at low concentrations of HDL (36). Japanese people have polymorphisms that include PON1 584A->G and 172T->A (37). In conclusion, to monitor susceptibility from pesticides exposure and other chemicals on the job, gene-gene and gene-environmental interactions need to be further investigated. Agirbasli suggests that diseases dealing with the circulatory system should assess multiple gene-gene interactions and their cellular pathways and environmental-gene impact.

This study had a few limitations. One limitation of the study is that the specific pesticides the participants were exposed to are not known. Also, the group with a medium intensity of exposure to pesticides had a small sample size so it was combined with low pesticide exposure. Most participants were in the no exposure group. Participants exposed to pesticides were exposed to a wide range of chemical types and classes. Future studies should look at the combination of both pesticides and solvents occupational exposure in relation to developing CVD, which might increase the number of outcomes. They should look at the combination of both exposure because those two chemical types were associated with mortality from CVD in a previous study of the same cohort. Workers on job could be potentially exposed to multiple chemicals everyday depending on the type of labor and task involved. Limited generalizability of pesticides (independent variable) are effected by other factors, which are the risk factors in the adjusted models, thus, effecting the results of the outcome (dependent variables of cardiovascular disease)..

Study advantages included a large sample size and wealth of information from previous studies published from the same study cohort. Another strength of this study is the fact that the whole cohort is men because some pesticides affect men in a different way than women, and

some don't affect women at all (15). Another advantage is that our population consists of individuals from the same ethnic group. This is a highly homogeneous group and allows for fewer genetic influences.

Chapter 4 : Conclusion of Findings

Multiple risk factors contribute to the development of cardiovascular diseases, including chemical exposure both from occupation and lifestyle choices. Chemical exposure factors potentially affects intermediate risk factors for developing CVD. During the literature review, gender, pesticide chemical type, and dosage played a bigger role than previously thought. Although sample size for high exposure was small compared to the unexposed group of the cohort, high pesticide exposure was still associated with developing cardiovascular disease. Employees can be exposed to these chemicals on the job 10 to 20 years prior and because of the long half-lives of these chemicals, the effect can still be seen leading to potential association of disease. By looking at different lag times of the population cohort, we were able to see when the effects of exposure started taking place. More research needs to be done on the difference between gender and occupational pesticide exposure and development of diseases. It was an asset that ethnicity and gender were the same in this study of pesticide occupational chemical exposure due to the gender differences found in toxicity and clinical drug trails. In addition, it provided a more homogeneous group which facilitates studying these associations.

This study provided some valuable insight into chemical occupational exposure and incident cardiovascular diseases and it is consistent with the study by Hung from Taiwan that suggests that acute high-dosages of pesticide exposure, consumed by mouth to attempt suicide, may contribute to development of CVD among survivors.

Future studies of genetic polymorphisms of the PON genes (1,2,3) and of gene-environmental interactions need to be further explored in our cohort to determine if there is an association between pesticide exposure and oxidative stress. Some polymorphisms of the PON1 gene have stronger associations with cardiovascular diseases than others. This study is the first of its kind to assess relative risk of intermediate risk factors such as blood pressure, in relation to pesticide exposure and CVD together. It is also thought that as individuals age, the effects of chemical exposures may be masked by other factors.

The findings of this research provide insight in the harmful effects that pesticides have on the cardiovascular system and confirms a positive association between high levels of pesticide exposure and CVD incidence. These data are helpful in identifying groups of subjects, such as those involved in agriculture and manufacturing of pesticides that may be at high risk of developing CVD. In addition, it highlights the importance of measures adopted by NIOSH, such as protective gear, to limit occupational exposure to pesticides, to reduce the increased risk of developing CVD and other diseases associated with pesticide exposure.

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Appendix

Risk Factors at Baseline For Those With and Without Incident Cerebro-Vascular Accident

variables	NO CVA n=6622	CVA n=935	p-value
age	54.17 ± 5.50	55.53 ± 5.74	<.0001
BMI	23.78 ± 3.11	24.10 ± 3.11	0.0032
SBP	132.78 ± 20.44	140.54 ± 21.74	<.0001
DBP	81.66 ± 11.38	85.40 ± 12.44	<.0001
Cholesterol	217.93 ± 37.77	216.93 ± 39.29	0.4478
Triglyceride	234.99 ± 204.10	245.17 ± 199.04	0.1612
Glucose	159.81 ± 56.94	169.47 ± 64.15	<.0001
Exercise	32.90 ± 4.55	32.91 ± 4.42	0.948
Smoking	23.51 ± 24.19	25.44 ± 26.00	0.0251
Alcohol	13.84 ± 24.31	15.56 ± 26.65	0.0467
Education	49.74 ± 50.00	45.35 ± 49.81	0.012

Cox Proportional Hazard Models of Pesticide Exposure in the First Ten Years For Incident Cerebro-Vascular Accident

Variables	Low-Moderate HR (95% CI)	p-value	High HR (95% CI)	p-value
Unadjusted	0.67 (0.17-2.69)	0.569	1.73 (1.10-2.71)	0.018
Age	0.61 (0.15-2.46)	0.487	1.35 (0.86-2.14)	0.191
Risk factors no BMI or SBP	0.61 (0.15-2.45)	0.485	1.45 (0.90-2.33)	0.123
Risk factors + SBP only	0.47 (0.12-1.91)	0.292	1.43 (0.89-2.30)	0.139
Risk factors + BMI only	0.60 (0.15-2.42)	0.471	1.45 (0.90-2.32)	0.127
All combined risk factors	0.47 (0.12-1.91)	0.292	1.43 (0.89-2.29)	0.14

Cox Proportional Hazard Model of Pesticide Exposure in the Thirty-Four Years
For Incident Cerebro-Vascular Accident

Variables	Low-Moderate HR (95% CI)	p- value	High HR (95% CI)	p- value
Unadjusted	0.67 (0.17-2.69)	0.569	1.73 (1.10-2.71)	0.018
Age	0.61 (0.15-2.46)	0.487	1.35 (0.86-2.14)	0.191
Risk factors no BMI or SBP	0.61 (0.15-2.45)	0.485	1.45 (0.90-2.33)	0.123
Risk factors + SBP only	0.47 (0.12-1.91)	0.292	1.43 (0.89-2.30)	0.139
Risk factors + BMI only	0.60 (0.15-2.42)	0.471	1.45 (0.90-2.32)	0.127
All combined risk factors	0.47 (0.12-1.91)	0.292	1.43 (0.89-2.29)	0.14

Risk Factors at Baseline For Those With and Without Incident Coronary Heart
Disease

Variables	NO CHD n=5695	CHD n=1882	p-value
Age	54.39 ± 5.58	54.18 ± 5.48	0.1714
BMI	23.59 ± 3.12	24.53 ± 2.97	<.0001
SBP	131.97 ± 20.17	139.08 ± 21.59	<.0001
DBP	81.22 ± 11.42	84.85 ± 11.66	<.0001
Cholesterol	214.84 ± 37.08	226.80 ± 39.17	<.0001
Triglyceride	225.95 ± 189.29	267.45 ± 238.84	<.0001
Glucose	158.41 ± 55.48	168.86 ± 64.29	<.0001
Exercise	32.96 ± 4.54	32.74 ± 4.51	0.0668
Smoking	23.62 ± 24.65	11.01 ± 21.18	0.4407
Alcohol	15.07 ± 25.58	11.01 ± 21.18	<.0001
Education	48.55 ± 49.98	51.14 ± 50.00	0.0512

Cox Proportional Hazard Model Pesticide Exposure in the First Ten Years for
Incident Coronary Heart Disease

Variables	Low-Moderate	p-	High	p-value
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	HR (95% CI)	value	HR (95% CI)	
Unadjusted	0.48 (0.16-1.51)	0.211	1.26 (0.88-1.82)	0.21
Age	0.47 (0.15-1.45)	0.187	1.15 (0.80-1.66)	0.454
Risk factors no BMI or SBP	0.48 (0.15-1.48)	0.2	1.27 (0.87-1.85)	0.223
Risk factors + SBP only	0.45 (0.15-1.41)	0.173	1.30 (0.89-1.90)	0.17
Risk factors + BMI only	0.47 (0.15-1.46)	0.189	1.27 (0.87-1.86)	0.214
All risk factors combined	0.45 (0.14-1.41)	0.17	1.31 (0.90-1.91)	0.165

Cox Proportional Hazard Model Pesticide Exposure for Thirty -Four Years for Incident Coronary Heart Disease

Variables	Low-Moderate HR (95% CI)	p-value	High HR (95% CI)	p-value
Unadjusted	1.07 (0.74-1.56)	0.709	1.04 (0.86-1.27)	0.655
Age	1.04 (0.71-1.51)	0.851	0.97 (0.80-1.18)	0.75
Risk factors no BMI or SBP	0.96 (0.65-1.42)	0.853	1.01 (0.82-1.23)	0.948
Risk factors + SBP only	0.92 (0.63-1.36)	0.683	1.02 (0.83-1.24)	0.873
Risk factors + BMI only	0.95 (0.64-1.40)	0.78	1.01 (0.82-1.23)	0.955
All risk factors combined	0.92 (0.62-1.35)	0.655	1.02 (0.83-1.24)	0.874